# CYTOCHROME OXIDASE AND OXIDATION OF CO IN EGGS OF THE SEA URCHIN STRONGYLOCENTROTUS PURPURATUS [1]

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Black and Tyler (1959) have shown that developing eggs of the sea urchin and the gephyrean worm *Urechis* can oxidize CO, and these authors have presented data on the inhibitory effects of CO on respiration of developing embryos. The rates of CO-oxidation and ordinary respiration were such that in the presence of CO an excess gas-uptake occurred in the light in early developmental stages, but the percentage of excess uptake diminished as the respiratory rate increased. In the dark there was increasing inhibition of respiration and, in *Urechis,* diminishing CO-oxidation as the respiratory rate increased.

The inhibitory effect of CO on cytochrome oxidase is well-known. Fenn and Cobb (1932) were the first to suggest that cytochrome oxidase could also oxidize this inhibitor, and Breckenridge (1953) obtained direct proof of this phenomenon, using labelled CO and purified cytochrome oxidase from pig heart. In the latter study, the author suggested a relationship between the relative proportions of oxidized and reduced iron atoms of cytochrome oxidase and the efficiency of CO-oxidation.

In order to interpret the data obtained on CO-oxidation in embryos, it was necessary to determine whether cytochrome oxidase was in the pathway of CO-oxidation in this material, and if so, whether the rates of CO-oxidation in light and dark by enzyme preparations could be changed by altering the amounts of cytochrome *c* in the system. It will be shown that cytochrome oxidase is involved in CO-oxidation in sea urchin eggs, and that the relative rates of oxygen-uptake and CO-oxidation can be altered by changing the concentration of cytochrome *c*.

## Materials and Methods

Granular preparations of active cytochrome oxidase were made by centrifugation of homogenates of unfertilized, jelly-free eggs of *Strongylocentrotus purpuratus* in 0.1 *M* phosphate, made to pH 7.4 with "$CO_2$-free" NaOH. The eggs were repeatedly forced through a No. 18 needle into 30 volumes of buffer, and the homogenate was transferred quantitatively to centrifuge tubes. The preparation was centrifuged at approximately 44000 × gravity in an angle head of the Spinco ultracentrifuge for 20 minutes. The sedimented granules were re-suspended in fresh buffer and centrifuged again at the same acceleration. The final sediment was suspended in 0.1 *M* buffer, pH 7.4, to give a concentration of 10%, based on

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original egg volume. Quantitative transfers of homogenates, combined with counts made on aliquots of the original egg suspension, made it possible to base the values obtained for cytochrome oxidase activity and CO-oxidation on egg-numbers, since it could be assumed that a constant percentage of the total activity was recovered in separate runs.

The enzyme activities were measured for one hour in Warburg vessels of the type devised by Stanley and Tracewell (1955), and the $CO_2$ was measured and recovered for $C^{13}/C^{12}$ analysis. The procedures involved in the measurement of gas-uptake and CO-oxidation have been described (Black and Tyler, 1959). The vessels were illuminated in these experiments by a bank of 150-watt G.E. floodlamps, providing an intensity of 3500 to 5000 foot candles at the level of the enzyme suspensions. In the dark experiment, the measurements were made at night.

TABLE I

*Rates of CO-oxidation by granule-preparations in the presence and absence of cytochrome c and ascorbate. Final concentrations, where appropriate, were as follows: egg granules, 6% (based on egg volumes); cytochrome c, $2 \times 10^{-4}$ M; ascorbate, 0.02 M. All vessels contained $PO_4$, 0.06 M, pH 7.4, and $AlCl_3$, $8 \times 10^{-4}$ M. Experiments were run in light.*

| Experiment | Contents of vessel | CO oxidized ($mm.^3/10^6$ eggs/hr.) |
|---|---|---|
| 1 | Ascorbate + cytochrome *c* (blank) | 1.3 |
| | Granules only | 2.4 |
| | Granules + ascorbate | 5.5 |
| | Granules + ascorbate + cytochrome *c* | 11.3 |
| 2 | Granules + cytochrome *c* | 3.2 |
| | Granules + ascorbate | 4.8 |
| | Granules + ascorbate + cytochrome *c* | 20.0 |

## RESULTS

The rates of CO-oxidation by two granule-preparations in the presence and absence of excess ascorbic acid and excess cytochrome *c* are presented in Table I. It can be seen that rates of CO-oxidation are very low in preparations lacking either ascorbate or cytochrome *c*. A low rate of CO-oxidation was also found in the blanks containing ascorbate and cytochrome *c*.

In the presence of both cytochrome *c* and ascorbic acid, rates of CO-oxidation comparable to those of unfertilized eggs and early developmental stages are found. It may be concluded that both these substances are necessary for the efficient oxidation of CO by the granule-preparations.

In Table II, the rates of gas-uptake and CO-oxidation in light and dark are listed for enzyme preparations at three different concentrations of cytochrome *c*. It can be seen that at cytochrome concentrations giving an $O_2$-uptake of ½ to ¼ the maximum rate, the rate of CO-oxidation in the light is actually higher than that in the vessels containing excess cytochrome *c*. Furthermore, in those vessels in which cytochrome *c* is limiting the rate of $O_2$-uptake, there is an excess gas-uptake in CO in the light. In the presence of excess cytochrome *c* the $O_2$-uptake is inhibited by CO in the light. It is of interest that Breckenridge (1953) also obtained excess gas-uptake in CO by his cytochrome oxidase preparations. In the dark the $O_2$-uptake is strongly inhibited by CO at all three concentrations of

cytochrome *c*, but the percentage inhibition is greatest at high concentrations of cytochrome *c*. There is not much difference between the rates of CO-oxidation in the dark at different cytochrome concentrations.

## Discussion

It can be concluded from the data of Tables I and II that added cytochrome *c* is necessary for the oxidation of CO by cytochrome oxidase, but the concentration of cytochrome *c* which gives maximum $O_2$-uptake is higher than the optimum concentration for CO-oxidation in the light.

Table II

*Rates of gas-uptake and CO-oxidation by granule preparations in the light and dark. All rates are corrected for the autoxidation in the ascorbate-cytochrome c blanks. The vessels contained $PO_4$, 0.06 M, pH 7.4; $AlCl_3$, $8 \times 10^{-4}$ M; ascorbate, 0.02 M, and homogenate, 6% (based on egg volumes). Final concentrations of cytochrome c are given below.*

| Experiment | Cytochrome *c* concentrate in moles/liter | Gas-uptake ($mm.^3/10^6$ eggs/hr.) | | | | CO oxidized ($mm.^3/10^6$ eggs/hr.) | |
|---|---|---|---|---|---|---|---|
| | | Light | | Dark | | Light | Dark |
| | | Air | 80% $CO/O_2$ | Air | 80% $CO/O_2$ | | |
| 1 | $2 \times 10^{-4}$ | 762 | 462 | | | 16.3 | |
| | $4 \times 10^{-5}$ | 364 | 382 | | | 26.0 | |
| | $2 \times 10^{-5}$ | 187 | 254 | | | 21.4 | |
| 2 | $2 \times 10^{-4}$ | 779 | 495 | 639 | 61 | 16.8 | 6.1 |
| | $4 \times 10^{-5}$ | 356 | 408 | 357 | 139 | 26.5 | 6.1 |
| | $2 \times 10^{-5}$ | 192 | 244 | 217 | 125 | 24.0 | 6.3 |

The data are of some value in interpreting the changes in CO-oxidation by developing eggs. Black and Tyler (1959) have postulated that in the light, an increasing rate of electron-transfer by the cytochrome system would enhance CO-oxidation up to a point. A comparison of rates of CO-oxidation in the light and no added cytochrome (Table I) with that when there is enough added cytochrome to give ½ maximum $O_2$-uptake (Table II) shows that the rate of CO-oxidation is increased about 5-fold by this increase in electron transfer. However, at very high rates of activity (excess cytochrome *c*) the rate of CO-oxidation falls. This is in accord with the suggestion of Breckenridge (1953) that CO would inhibit cytochrome oxidase (and presumably CO-oxidation) when a relatively high proportion of the iron of cytochromes *a* and $a_3$ became reduced, as it might in the presence of excess cytochrome *c* and excess reducing substrate. It can be seen from Table II, column 4 that in the presence of excess cytochrome *c* there is inhibition of $O_2$-uptake by CO in the light.

The similarity between the excess gas-uptake in CO in the light exhibited by the enzyme preparations at low concentration of cytochrome *c* (Table II), and the gas-uptake in CO by whole eggs during early development is worth noting. For the whole eggs, Black and Tyler regarded the results as an indication that

during early development the cytochrome oxidase was "unsaturated" with reducing substrate, as proposed originally by Runnström (1930) on the basis of other experiments. The present finding is in accord with that interpretation.

In the dark, the lack of variation in rate of CO-oxidation at different levels of cytochrome *c* is perhaps not too surprising, when the data are compared with those for intact embryos of the sea urchin, in which the rate of CO-oxidation changes little during development. It should be noted that even at the lowest levels of cytochrome used in the dark, the rate of $O_2$-uptake by the granules was still much higher than that of early, developing eggs. Presumably if the cytochrome *c* had been reduced still further in these preparations, an excess gas-uptake in CO might have been evident in the dark, as it was in unfertilized and fertilized eggs.

## Summary

1. The cytochrome oxidase activity and ability to oxidize CO (labelled with $C^{13}$) have been determined for sedimented, washed particles obtained from unfertilized eggs of the sea urchin, *Strongylocentrotus purpuratus*. Both added cytochrome *c* and ascorbate were required for the rapid oxidation of CO by the particles, and it is concluded that cytochrome oxidase is in the pathway of oxidation.

2. In the light, the rate of CO-oxidation was found to be greatest at the concentration of cytochrome *c* which gave about ½ the maximum $O_2$-uptake. When the uptake of oxygen was maximal, the CO inhibited both the $O_2$-uptake and the CO-oxidation in the light. In the dark the rates of CO-oxidation by the particles were not greatly affected by changes in the level of cytochrome *c*. The results are used as a basis for the interpretation of developmental changes in CO-oxidation in embryos.

## LITERATURE CITED

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